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IN VITRO EVALUATION OF THE SYNERGISTIC ACTIVITY OF NEOMYCIN-POLYMYXIN B ASSOCIATION AGAINST PATHOGENS RESPONSIBLE FOR OTITIS EXTERNA

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The most recent guidelines recommend, for otitis externa antibiotic therapy, the use of topical formulations in that they are very safe, have a quicker effect and do not induce bacterial resistance compared to systemic therapy. The choice of the class of antibiotics in empiric therapy of otitis externa must take into consideration the polymicrobic nature of the infection that includes both bacteria (Gram-positive and Gram-negative) and mycetes. For this reason, in this study we evaluated the synergic activity of neomycin in association with polymyxin B against the pathogens commonly responsible for otitis externa, compared to that of a single antibiotic (ciprofloxacin). The polymyxinB/neomycin association shows clear synergic effects with values of both Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) reduced by 3-4 times with respect to the single antibiotic; and in *P. aeruginosa* the synergistic effect of the neomycin/polymyxin B association with respect to neomycin was more evident (5-6 times), with an intrinsic *in vitro* activity constantly higher than that of ciprofloxacin alone or in association with hydrocortisone. From the analysis of the data obtained *in vitro*, we can conclude that the possibility of using a topical formulation containing a synergistic association of antibiotics, such as neomycin-polymyxin B, in such a way as to obtain the maximum effect in the minimum time with an increase in the spectrum of action of non-bacterial pathogens, is an optimal choice for the clinician for the empiric therapy of otitis externa.

Otitis externa is an inflammation of the outer ear canal and tympanic membrane, which has become one of the most frequent ENT pathologies. In most cases it is acute, sometimes associated with otitis media or infections of the upper respiratory tract and, in rare cases, there are severe complications (periauricular abscess, perichondritis) (1). It is therefore important that the clinician obtains a rapid eradication of the pathogens responsible by means of a therapy based on cleansing the canal and administering antibiotics (oral or topical).

In its acute form, the etiology of otitis externa includes both bacteria (Gram-positive and Gram-negative) and mycetes; even if data in the international literature suggest that a principal role is played by *Pseudomonas aeruginosa* (30-50%), *Staphylococcus aureus* (10%) and *Candida albicans* (10%) (2).

The therapeutic choice for otitis externa is, in most cases, empirical and must thus take into

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consideration the spectrum of activity of the antibiotic against most of the pathogens involved; this is not always possible using a single antibiotic given the polymicrobic nature of the infection. For this reason, in this study we evaluated the synergistic activity of the neomycin-polymyxin B association: these are the active agents commonly used for the treatment of acute and chronic otitis externa, with the addition of lidocaine, a local anesthetic (3-4).

Neomycin, an aminoglycosidic antibiotic, has a wide spectrum of action including Gram-positive cocci and bacilli (above all staphylococci) and Gram-negative bacteria (*Enterobacter* spp., *Klebsiella* spp., *Serratia* spp., *P. aeruginosa* and *Proteus* spp.).

Polymyxin B is a cyclic peptide, principally active against *P. aeruginosa, Klebsiella spp., Enterobacter* spp., *Escherichia coli* and *Haemophilus influenzae.* Its activity also extends to some fungal species such as *Candida* spp.

The association of neomycin with polymyxin B is thus active against all the bacteria commonly responsible for the various ENT forms of infection, in particular *P. aeruginosa, S. aureus* and *Candida* spp., principal etiological agents of otitis externa in children and adults.

MATERIALS AND METHODS

The evaluation of the synergistic activity of neomycin and polymyxin B, compared to ciprofloxacin, was carried out both in terms of intrinsic capacity Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) on all the strains examined, and in terms of bactericidal velocity against *P. aeruginosa* and *S. aureus*, which are the pathogens principally responsible for otitis externa (5). The eventual interference of lidocaine (with the neomycin/polymyxin B association; Anauran®) and hydrocortisone (with ciprofloxacin; Mediflox®) was also evaluated against *in vitro* anti-microbial activity.

Strains

The following 144 clinically isolated strains were tested: 64 Gram-positives (32 *S. aureus*, 6 *S. epidermidis*, 2 *S. xylosus*, 2 *S. hominis*, 12 *S. pneumoniae* and 10 *S. pyogenes*) and 70 Gram-negatives (50 *P. aeruginosa*, 8 *E. coli* and 12 *K. pneumoniae*), as well as 10 strains of Candida (*C. albicans, C. parapsilosis* and *C. krusei*).

Control strains were: *S. aureus* ATCC 29213, *E. coli* ATCC 35218, E. coli ATCC 25922, P. aeruginosa ATCC 27853, *S. pneumoniae* ATCC 49619, *S. pyogenes* DSM 20565, *C. albicans* ATCC 90028, *C. krusei* ATCC 6258, and *C. parapsilosis* ATCC 22019.

All strains were seeded and purified in their respective culture media (MacConkey for the Gram-negatives, MSA for the staphylococci, CNA for the streptococci and Sabouraud for the mycetes).

The determination of the MIC, the MBC and the bactericidal curves for *S. aureus* and *P. aeruginosa* were assayed for neomycin, polymyxin B, ciprofloxacin, as well as the "neomycin-polymyxin B-lidocaine" and "ciprofloxacin-hydrocortisone" associations.

Table I. *MICs and MBCs of 5 antimicrobial agents against 144 strains of Gram positive and Gram negative pathogens.*

Drug	MIC_{50}	MIC_{90}	MBC_{50}	MBC_{90}
Ciprofloxacin	0.25	32	0.5	64
Cipro/Hydro	0.5	64	2	256
Neomycin	16	128	16	128
Polymyxin B	1	64	1	128
Neo/Poly B	1	32	2	32

Table II. *Synergistic effect of polymyxin/neomycin combination on S. aureus and P. aeruginosa.*

Drug	*S. aureus*		*P. aeruginosa*	
	MIC_{90}	MBC_{90}	MIC_{90}	MBC_{90}
Ciprofloxacin	4	4	32	64
Cipro/Hydro	8	8	32	256
Neomycin	128	128	64	128
Polymyxin B	128	128	1	2
Neo/Poly B	8	8	2	2

Table III. *MICs of 3 antimicrobial agents against 10 strains of Candida spp.*

Strain	MIC (ug/ml)		
	Poly B	Neo/Poly B	Ciprofloxacin
C.parapsilosis	32	16	>512
C.albicans	32	16	>512
C.kruseii	16	8	>512

neomycin/polymyxin B association with respect to neomycin was more evident (5-6 times), with an intrinsic *in vitro* activity constantly higher than that of ciprofloxacin alone or in association with hydrocortisone.

Finally, the anti-fungal activity of polymyxin B (Table III) was confirmed against all the strains of Candida tested, and we show that the association with neomycin caused, in some cases, a slight reduction in the MIC values. Obviously, ciprofloxacin had no anti-fungal activity. Figs. 1 and 2 show the results relative to the bactericidal curves. In *S. aureus* (Fig. 1) there is an overlap of the curve of the neomycin/polymyxin B association with that of ciprofloxacin, with a reduction in the bacterial concentration after 2 h and a complete removal at 8 h. A better bactericidal effect of the association with respect to ciprofloxacin is, instead, already found in *P. aeruginosa* at 2 h and is still greater at 4 h and 8 h (Fig. 2). It must also be said that from the observation of the bactericidal curve the synergic action between polymyxin B and neomycin is confirmed.

DISCUSSION

The most recent guidelines (6) recommend, for antibiotic therapy of the uncomplicated form of otitis externa, the use of topical formulations in that they are very safe, have a quicker effect and do not induce bacterial resistance with respect to systemic therapy. Considering that, other than the bacterial component, also some species of Candida can be responsible, alone or in association with bacteria, for the onset of otitis externa, it thus follows that the therapeutic choice must be aimed at topical ear antibiotics with a wide spectrum of action, such as the neomycin-polymyxin B association, that, not only increases the antibacterial activity of the single components, but also extends, with respect to ciprofloxacin, the spectrum of action to mycetes.

These considerations have a valid theoretical support in the various mechanisms of action of the two agents: neomycin fixes at the level of the 30S subunit of the bacterial ribosome, inhibiting the exact incorporation of the aminoacids in the polypeptide chain, from which an incorrect protein synthesis derives. Polymyxin B, instead, fixes to the cytoplasmatic membrane of bacteria, altering its permeability, with the consequent loss of cellular constituents essential for the life of the organism.

The choice of the antibiotic class in empiric therapy of otitis externa must obviously take into consideration not only the spectrum of activity, but also the possibility of obtaining a bactericidal effect in as short a time as possible. The association of the antibiotic with hydrocortisone or analgesics, without interfering with the efficacy of the antibiotic, is useful for the possibility of obtaining a rapid resolution of the inflammatory process or pain. In our study we did not find any intrinsic anti-bacterial activity or interference effect of hydrocortisone and lidocaine.

From the analysis of the data obtained *in vitro*, we can conclude that the possibility of using a topical formulation containing a synergistic association of antibiotics, such as neomycin-polymyxin B, in such a way as to obtain the maximum effect in the minimum time with an increase in the spectrum of action of non-bacterial pathogens, is an optimal choice for the clinician for the empiric therapy of otitis externa.

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